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Ontogeny *Versus* Phylogeny in Determining Patterns of Chemoreception: Initial Studies with Fiddler Crabs

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Abstract. Fiddler crab (*Uca longisignalis*) first stage zoea and adults were assayed for behavioral responses to 16 amino acids and sugars. Larval chemosensitivity was examined using computer–video motion analysis of swimming behavior. Adult sensitivity was assayed by determining the substances that elicit feeding. The pattern of chemoreception expressed by *U. longisignalis* adults is strongly correlated with that measured previously in adult sand fiddler crabs, *Uca pugilator.* This concordance among abilities probably reflects shared trophic ecologies of the two species. In contrast, a quantitative analysis shows no significant correlation between the sets of compounds inducing chemoreceptive behavior by larval and adult *U. longisignalis.* The strongest responses (by both stages) are elicited by substances found in potential prey, and differences in prey types among larvae and adults appear responsible for the lack of correlation. Larvae do, however, respond to substances abundant in prey consumed by adults, even though these substances are absent, or occur at low levels, in larval prey. Adults, on the other hand, appear insensitive to compounds that cue only larval food, but which are maximally stimulatory to larvae. Consequently, our results indicate that the abilities of one life-history stage may be constrained, through development, by the requirements of later stages. The patterns of correlation among adults of different species, and among life-history stages within a species, indicate that both ecological context and developmental factors influence patterns of chemosensitivity.

Introduction

Studies on marine invertebrates have engendered a large body of literature on chemosensory properties and their ecological implications. Several broad scale patterns are apparent, based on current work. In general, the particular spectrum of substances eliciting a response is correlated with diet. Flesh eaters tend to respond strongly to amino acids, sometimes to organic acids and nucleotides, and are generally unresponsive to sugars (see reviews of Ache, 1982; Carr, 1988; Laverack, 1988; Zimmer-Faust, 1989). The particular substances generating large responses are often dominant organic components in prey tissues. In contrast to flesh eaters, herbivorous organisms are highly responsive to carbohydrates (Zimmer *et al.,* 1979; Robertson *et al.,* 1981; Trott and Robertson, 1984; Rittschof and Buswell, 1989), which occur as algal storage products and components of extracellular mucous sheaths (Fogg, 1966; Fogg *et al.,* 1973; Craigie, 1974; Darley, 1977; Paulsen *et al.,* 1978) and sometimes to amino acids that are characteristic of algal material.

In spite of broad scale associations between diet and the response spectrum of organisms, related or ecologically similar species can display quite dissimilar fine scale patterns of chemosensitivity. The spiny lobsters *Panulirus argus* and *P. interruptus* each respond to a wide array of amino and organic acids, and exhibit a broad overlap in the substances eliciting a response. However, within the overlapping subset of stimulatory substances, these two species are differentially responsive to the same compounds. For example, *P. argus* is most responsive to citric acid, ascorbic acid, succinic acid, taurine, and glycine (Ache *et al.,* 1978), whereas *P. interruptus* responds mostly to glycine, alanine, serine, succinic acid, oxalic acid, and adenosine triphosphate (Zimmer-Faust *et al.,* 1984; Zimmer-Faust, 1987). Similarly, the coexisting copepods *Acartia hudsonica* and *Eurytemora herdmani* both respond to amino acids, although *Acartia* is stimulated mostly by aliphatic amino acids (*e.g.,* leucine and valine) whereas *Eurytemora* is most responsive to the dicarboxylic

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amino acids (*i.e.*, glutamic and aspartic acid). When responses to the same compounds are observed, each species reacts to them in different ways (Poulet and Ouellet, 1982). *Acartia*, for instance, is repelled by aspartic acid, while *Eurytemora* is attracted to it.

To date, explanations of chemosensory abilities have focussed largely on adaptive hypotheses. It is implicitly assumed that the response magnitude varies directly with the relative concentration of substances in prey, thus allowing animals to locate potential food items more efficiently. Other explanations exist, especially where organisms progress through a series of life-history stages requiring different chemosensory abilities. At any one stage, abilities (1) may be functionally important, (2) may represent incipient abilities required at a later stage, or (3) may be relictual abilities that were required by previous life-history stages. Developmental explanations for patterns of chemosensitivity may be especially important for marine organisms where differences in the habitats occupied by larval and adult stages can be large.

Fiddler crabs (genus *Uca*) provide an excellent system with which to quantitatively investigate correlations of abilities among species, and life-history stages. All adult fiddler crabs are deposit feeders, foraging on intertidal sediments (Crane, 1975). Trophic habits are quite uniform (Teal, 1958; Miller, 1961; Crane, 1975; Robertson *et al.*, 1980; Weissburg, 1990), and several species often coexist within a limited geographic area (Crane, 1975). Further, adult and larval habitats are markedly different in terms of abundance levels, and specific components of the chemical environment (*e.g.*, Degens *et al.*, 1964; Andrews *et al.*, 1971; Clark *et al.*, 1972; Mopper *et al.*, 1980; Mopper and Lindroth, 1982).

The investigation reported here was designed to explore the potential influence of both ecological and developmental processes on adult and larval chemosensitivity. The logical starting point for detecting developmental constraints on chemoreception is to determine patterns of chemosensitivity at the endpoints of the ontogenetic series. Similarly, the concordance of chemosensory abilities in at least one pair of species with similar trophic habits should be established before a detailed survey of ecologically and taxonomically related species is attempted. Therefore, we have examined, quantitatively, the chemosensory abilities of first stage zoea and adult stages of the crab, *Uca longisignalis*, and have compared the properties of different life-history stages in the same species, and among adults of *U. longisignalis* and *U. pugilator.*

Materials and Methods

Preparation of test solutions

Metabolites released by Artemia salina *in culture.* An initial experiment was performed to explore larval swimming behavior in response to substances released by a documented prey species. The solution was prepared from a culture of young brine shrimp (*Artemia salina*)—a standard food source used in rearing carnivorous larvae of marine invertebrates, including fiddler crabs (*e.g.*, Herrnkind, 1968; Rabalais and Cameron, 1983; Christy, 1989). *Artemia* nauplii were hatched from cultures incubated in 20 ppt artificial seawater media (ASW) (Forty Fathoms Marinemix) prepared with HPLC grade deionized water. When nauplii were 1–3 h old, 100 ml of the batch culture were spun down in a Beckman (Model J2-21) centrifuge at 10,400 × *g* and 2°C for 30 min. The resulting supernatant was decanted, filtered through a 0.45 μm membrane filter, diluted to a concentration of 15 ppt, then stored at −87°C.

Amino acids and sugars. Chemicals were prepared in ASW media at a concentration of 15.0 ppt, using HPLC grade deionized water. The following amino acids were used as test compounds: β-alanine (ALA), arginine (ARG), cysteine (CYS), glutamic acid (GAD), glycine (GLY), proline (PRO), taurine (TAU), and valine (VAL). The following sugars were used: galactose (GAL), glucose (GLU), maltose (MAL), sorbose (SOR), sucrose (SUC), and trehalose (TRE). These compounds were selected because they are important chemical constituents in either plant or animal components of the diet of marine zooplankters (*e.g.*, Jeffries, 1969), or have been found stimulatory to other deposit feeding crustaceans (*e.g.*, Robertson *et al.*, 1981; Rittschof and Buswell, 1989). Except where noted, all amino acids were L-isomers, and all sugars D-isomers. Chemicals were all reagent grade, obtained from the Sigma Chemical Company. Test solutions were prepared in single batches at a concentration of 10^{-4} *M* for experiments performed using larvae, or 10^{-1} *M* for experiments performed using adults, divided into 20 ml aliquots, and stored at −87°C until needed. Where necessary, solutions were adjusted to pH 8.0 using 1 *M* NaOH or HCl.

Experiments testing larval chemosensory abilities

Gravid *Uca longisignalis* females, collected from Airport Marsh, Dauphin Island, Alabama, were held until their broods hatched. Females were placed in finger bowls containing a small amount of filtered 15 ppt seawater, and kept in incubators at 26°C under a 14:10 light-dark cycle. Females were checked for brood release twice per day, and water was changed daily. Usually several females simultaneously released larvae, and these broods were combined, and held in filtered seawater at densities of about 10 larvae/ml prior to assaying chemosensory behavior.

Experiments on larval chemosensory abilities began 24–36 h after hatching and ran for 24 h. We specifically restricted our experiments to these early stages because larvae are only competent to settle on substrates and metamorphose to the adult form later in the developmental series, after they have reached the megalopa stage (*e.g.*, Epifanio *et al.*, 1988). Therefore, behavior in response to test compounds cannot be ascribed to substrate selection and settlement processes. Twenty larvae were transferred in 5 ml of ASW into plexiglass microcosms (3 cm long × 3 cm wide × 5 cm high), and 20 ml of the 10^{-4} *M* test solution was gently added. After about 30 s of gentle stirring to disperse the test compound, the microcosm was placed into a darkened chamber, and larval swimming behavior was recorded. Each trial was video-recorded for a 10-min period. Order of the test solutions was determined by a random numbers table, subject to the constraints that: a specific compound was not presented twice in succession; and that seawater treatments were performed after every block of five test solutions. Five to eight replicate trials of each test compound were performed. Individual larvae were tested in only one trial and then discarded.

Larval swimming behavior in the horizontal plane was filmed using a Sony infrared-sensitive CCD camera (model HVM-200) equipped with a Tamron 180 mm lens, mounted beneath the microcosm, and focused 1.0 cm (25 to 35 larval body lengths) above the bottom. Similarly sized chambers and techniques have been successfully used for video recording locomotory behavior of small zooplankters, including copepods (Buskey, 1984), tintinnids (Buskey and Stoecker, 1989), and polychaete (Butman *et al.*, 1988) and molluscan larvae (Zimmer-Faust and Tamburri, in prep.). The chamber was illuminated with an infrared light source (>830 nm) oriented 90° to the axis of the video camera. The field of view was 6.9 × 6.8 mm, about 6.4% of the total cross-sectional area of the microcosm. Depth of field was determined by mounting a pipette (tip diameter = 300 μm) on a micromanipulator calibrated in 10 μm increments. Using the computer-video motion analyzer (see below) with gray scale threshold set for discriminating larvae, the pipette tip was moved vertically to determine the limits at which the analyzer would 'recognize' the tip as an object. The recognition distance was 1.5 mm in the vertical dimension.

Quantification of swimming speed and turning behavior was accomplished by replaying each video tape through a computer—video motion analysis system (Motion Analysis Corp. Model VP 110 and ExpertVision software package) interfaced with an Amdek 386 microcomputer. The system constructs a digitized record of the raw video data, using a gray scale detector to enhance the contrast between objects in the video field and background. The outlines of the objects are defined, and the x,y coordinates of the centroid (geometric center) are calculated. The path of an object is reconstructed, on a frame-by-frame basis, as the translational movement through space of an object's centroid (see Buskey and Stoecker, 1989; Zimmer-Faust and Tamburri, in prep.). The video sampling rate of the motion analysis hardware was set at 10 frames/s, and, based on preliminary results, only the first 5 min of each trial were used in the analysis.

Analysis of larval behavior consisted of determining swimming speed and net-to-gross displacement ratio (NGDR) for each larval swimming path. The NGDR is the ratio of the linear distance between the starting and ending points (net distance) and the total distance traversed by the path (gross distance). The NGDR measures the tendency of paths to be circular or twisted, and reaches a maximum value of 1.0 for paths that are completely straight. An NGDR of zero implies a looped or circular path, with the origin and endpoint occurring at the same spatial coordinates. For each test substance, data for all paths were pooled across trials, and means compared to swimming behavior observed in seawater using a Student's *t*-test. Experiments exposing larvae to *Artemia* culture water and test compounds indicated mean speeds, and NGDRs did not differ significantly among paths during the 5-min analysis period.

Based on initial findings, glucose, valine, and trehalose were selected for dose-response trials because they were among the compounds producing the largest changes in larval swimming behavior. Using the above protocol, larval swimming behavior was determined for larvae exposed to a series of half log-step dilutions from 10^{-4} to 10^{-6} *M*. Three replicate trials were run at each concentration, and within each dilution series the order of presentation was randomly determined. Seawater controls were run every fifth trial, as before. Solutions were prepared by serial dilutions of the primary stock used in the initial assays. Individual larvae were tested once, then discarded. Swimming speed and NGDR were regressed on concentration using SAS regression procedures (SAS Institute, Carey, North Carolina).

Experiments testing adult chemosensory abilities

Adult *Uca longisignalis*, also collected from Airport Marsh, were held in a 1 × 2 m sea table equipped with a continuous flow of filtered, UV sterilized seawater, and a sandy substrate allowing animals to construct burrows. Animals had continuous access to both shallow water (5 cm depth) and exposed sandy regions. Air temperature varied between 27–32°C, water temperature was 24–27°C at a salinity of 18–20 ppt. Animals were fed a diet of commercial fish food pellets, and the benthic diatom *Cylindrotheca closterium*. Forty-eight hours prior to experimentation, animals were removed from sea tables, and in small groups, isolated in bowls containing clean azoic substrate and 15 ppt ASW held at 26°C.

Chemosensory assays were conducted on individual adult crabs (mean carapace width: 18.4 ± 1.8 mm SD) exposed to one of the 16 amino acids or sugars used previously in tests with larvae. A 40-l aquarium was subdivided into three sections using opaque partitions, and the bottom lined with a layer of clean beach sand. The sand was sloped so that when approximately 200 ml of ASW was added, the back third of each section contained about 1 cm of standing water. This arrangement allows crabs access to water required in feeding (Miller, 1961), and crabs forage readily in this type of arena (Weissburg, 1990). A glass cover maintained humidity.

Each crab was presented with a food patch embedded in the sand at the front of the test section. The patch was a 2.3 cm diameter culture dish, containing 4.0 g substrate mixed with 3 ml of the test solution. Based on the work of Robertson *et al.* (1981), test solutions were prepared at 10^{-1} *M*, because this concentration proved maximally stimulatory to adult *U. pugilator*. The parallel methodology employed by Robertson *et al.* (1981) and by us facilitates a quantitative comparison between the two studies. The substrate was mud, collected from a location at Airport Marsh where crabs were observed feeding. Sediment was washed through a 1 mm sieve, ashed overnight at 600°C, treated in hot 10 *M* HCl for 8 h, then washed repeatedly with HPLC-grade distilled water to remove all traces of acid. Feeding experiments took place at 26°C.

Crabs were allowed to feed for 2 h while their behavior was recorded on video tape, using a NEC black and white CCD video camera (Model Ti 22AII) equipped with a Tokina 35–70 mm macrofocal lens and 2× multiplier, connected to a JVC (Model BR 3200U) tape recorder and a Panasonic TR-124-MA video monitor. All three animals in the aquarium were visible, and at the front of the tank (where the patches were embedded), the image of the animals was magnified by approximately 25%, facilitating accurate analysis of feeding behavior. Each chemical was tested on 15 individuals of each sex.

The tapes were reviewed and the frequency of feeding induction was determined, where feeding was defined as more than three successive lifts of the chelae to the mouth in any 5-s interval. We chose this criterion to be conservative in distinguishing a feeding response from patch sampling behavior. We observed that 75–80% of the animals, including those tested on seawater controls, lifted a chelae to their mouth at least once during a trial, although none of the animals lifted its chelae more than three consecutive times in sediments containing only seawater. Fiddler crabs possess chemosensitive organs on the minor chelae (Robertson *et al.*, 1981), and probing is a mechanism to allow animals to determine the contents of each patch. Three consecutive lifts seems to discriminate feeding from sampling. Any animal that was not scored as sampling the patch was excluded from analysis. Approximately 75% of the animals met the criterion of exhibiting sampling behavior, and additional animals were tested as needed to produce sample sizes of 15 animals of each sex per compound. Thus, results cannot be considered artifacts of differences in sampling frequency among compounds. The total time spent feeding was also measured to indicate the intensity of the response, and was recorded to the nearest 1 s with the time counter on the video tape recorder.

Preliminary results indicated that males and females were similar in their responses, so the data from both sexes were pooled. A William's corrected *G*-test was used to determine whether the percentage of animals responding to a substance was greater than that of seawater controls. Time spent feeding was analyzed using a Student's *t*-test. Animals did not feed on the seawater controls, giving feeding times of 0.00 (see Results). Because feeding times on other compounds cannot be less than zero, this was a one-tailed *t*-test to determine whether feeding times were significantly greater than zero.

Comparing larval and adult chemosensory responses

Responses of larval and adult *Uca longisignalis* were quantitatively compared using a non-parametric correlation analysis. Kendal's Tau was computed based on the rankings of the significance tests of larvae and adults. Each stimulatory compound (for either life history stage) was ranked by the *t*-value (larvae) or *G*-value (adults). For larvae, the highest *t*-value from measurement of either NGDR or swimming speed was used (see Results). We ranked the *G*-scores for the adults, because, unlike the *t*-test, they are less affected by variation in the time at which an animal commenced feeding. If, for instance, an animal began feeding near the end of a trial, feeding times could be truncated as an artifact of the experimental trial length, whereas estimates of the percentage of animals feeding are immune to the timing of the response. In any case, results obtained using *t*-test scores differ little from an analysis done using the *G*-test scores (see Results). Because we are interested in comparing the subsets of chemicals stimulatory to each life-history stage, compounds producing no response in both larvae and adults were excluded from the correlation analysis.

Responses of adult *Uca longisignalis* and adult *Uca pugilator* were also quantitatively compared using Kendal's Tau. Rankings for *Uca pugilator* were taken from Robertson *et al.* (1981), giving the number of foodballs/animal produced by crabs foraging on a particular substrate and compound. The rankings for the stimulus intensity of each compound on *U. pugilator* were computed by averaging results from all trials performed presenting a particular compound. Several compounds were tested in only one experiment, and the confidence limits for the

Table I

Sample sizes and t-*values for* Uca longisignalis *larval swimming responses to chemical test solutions, relative to seawater controls*

Compound	Number of paths analyzed	*t*-value	
		Speed	NGDR
Artemia culture water	66	2.48**	2.97**
Amino acids			
β-alanine	108	2.86**	0.76
arginine	65	1.18	0.58
asparagine	120	1.14	1.77
cysteine	141	0.30	0.72
glutamic acid	40	0.77	0.76
glycine	145	0.68	1.19
proline	119	0.25	0.16
serine	109	0.41	0.23
taurine	150	0.49	1.77
valine	98	7.60***	3.76***
Sugars			
galactose	124	2.99**	0.37
glucose	114	3.72**	0.01
maltose	106	0.71	2.18*
sorbose	109	3.36**	0.16
sucrose	134	2.54**	0.61
trehalose	140	0.01	4.37***
Seawater	259	—	—

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

number of foodballs produced in response to these compounds overlapped. In this case, the rankings were considered ties.

Results

Experiments testing larval chemosensory abilities

Responses to metabolites released by Artemia *in culture.* Larvae responded to solutions derived from *Artemia* cultures by changing both swimming speed and turning behavior. Larval swimming speeds and NGDR's were significantly higher, relative to behavior in seawater (Table 1; Figs. 1, 2). We measured ammonia-N and dissolved organic carbon (DOC) in ASW and in culture water to assay for the presence of *Artemia* metabolites. The concentration of ammonia in culture water was 8.5 μM, and in ASW was 0.8 μM, as determined with an Alpkem nutrient autoanalyzer (Model RFA/2). Dissolved organic carbon occurred at 3.21 mg/l in culture water and at 0.75 mg/l in ASW, determined with a Shimadzu organic carbon analyzer (Model TOC-5000). The values obtained in culture water were typical for estuarine waters (Dame *et al.*, 1989; J. R. Pennock, pers. comm.) and clearly indicated the presence of *Artemia* metabolites.

Responses to amino acids and sugars. All of the sugars tested, as well as the amino acids valine and β-alanine, were stimulatory. Each substance, except for trehalose, caused changes in larval swimming behavior consistent with larval responses to *Artemia* culture water. Swimming speeds were increased in the presence of most sugars, and the two amino acids (Table 1; Fig. 1). The response to valine was the most dramatic, while responses to the other compounds were weaker. Galactose, glucose, sucrose, and β-alanine constituted a group producing roughly equal increases in swimming speed. Path trajectories were also changed in response to valine, maltose, and trehalose, as shown by the changes in NGDR (Table 1; Figs. 2, 3). Valine and maltose resulted in straighter paths, while larvae in trehalose solutions produced paths that were more circuitous (Fig. 3). For each of the other compounds listed in Table 1, but not appearing in Figures 1 or 2, mean swimming speeds were within ± 0.02 mm/s of that in

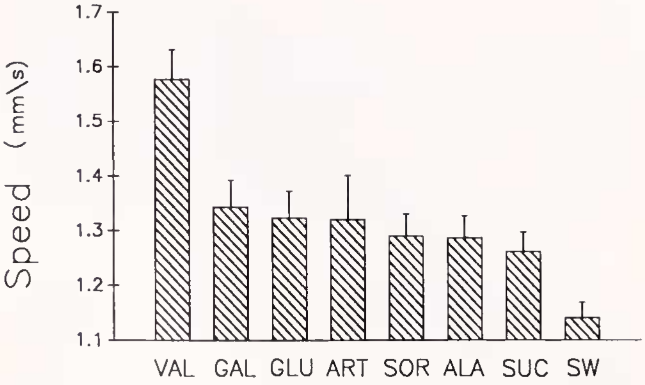

Figure 1. Mean swimming speeds (±1 SEM) of larvae exposed to test compounds at 10^{-4} *M*, metabolites released by *Artemia salina* (ART), and seawater. Data appear only for those compounds producing a significant change in larval swimming speed relative to seawater controls ($P < 0.05$; see Table I).

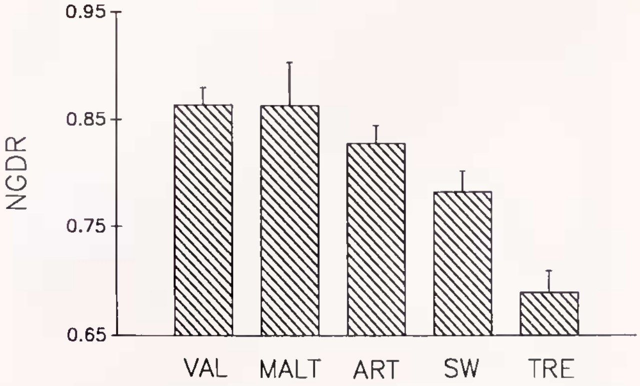

Figure 2. Mean NGDR (± 1 SEM) of larvae exposed to test compounds at 10^{-4} *M*, metabolites released by *Artemia salina* (ART), and seawater. Data appear only for those compounds producing a significant change in larval turning behavior relative to seawater controls ($P < 0.05$: see Table I).

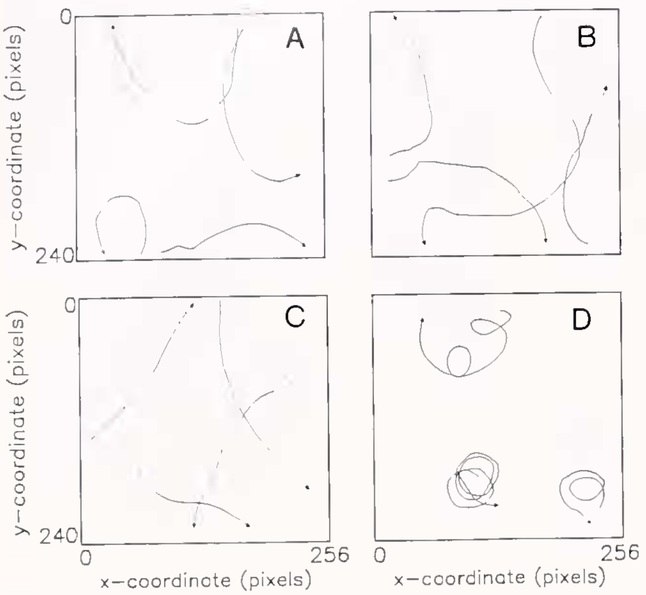

Figure 3. Typical swimming paths in the horizontal plane by larvae exposed to seawater and to test substances causing maximal responses. The x,y coordinates are in units of picture elements (pixels), where 1 pixel = 0.028 mm. All substances were tested at 10^{-4} *M*: A. seawater, B. glucose, C. valine, and D. trehalose. Relative to seawater, larvae swim faster in glucose, faster and straighter in valine, and turn more frequently in trehalose.

seawater, and *t*-tests yielded values of 0.0022–1.183 ($P > 0.20$, Table I). Mean NGDR values for non-stimulatory compounds were all within ± 0.02 of the NGDR in seawater, and *t*-values ranged from 0.020 to 1.77 ($P > 0.05$, Table I).

The dose-response curves for glucose, valine, and trehalose generally display a log-linear form (Fig. 4), allowing analysis by linear regression. There is a significant relationship between solution strength and response magnitude for all compounds. Responses to valine display the most sensitivity to dosage (slope = 0.097), followed by glucose (slope = 0.062), with responses to trehalose being least sensitive to dosage (slope = −0.048). The y-intercept of each curve is significantly different from seawater controls, according to a *t*-test (Fig. 4, $t \geq 8.94$, d.f. ≥ 229, $P < 0.01$, all comparisons). Larvae therefore respond to micromolar concentrations, and can probably respond to sub-micromolar stimulus concentrations as well. None of the regressions explain a great deal of the observed variance ($r^2 < 0.05$ in all cases), either due to the small slopes, or because additional factors may be important in determining larval swimming responses.

Experiments testing adult chemosensory abilities

Adult *Uca longisignalis* were induced to feed by a limited subset of the amino acids and sugars (Table II). The monosaccharide glucose, and the glucose-containing disaccharides, maltose and sucrose, induced the greatest percentage of animals to feed. Feeding was also elicited by the sugars, trehalose and sorbose, but to a much smaller degree, and the amino acids serine, glutamic acid, and β-alanine also provoked minor responses. Except for valine, which generated a non-significant increase in feeding, none of the other compounds elicited any behavioral response at all—*G*-values were 0.00 (Table II).

The feeding intensity elicited by each of the test compounds is also given in Table II, which lists foraging time for each treatment. All of the compounds that caused crabs to feed generated feeding times significantly greater than zero (one-tailed *t*-test) and, in general, the magnitude of response initiation and intensity was correlated. A Kendal's Tau, based on the rankings of feeding percent

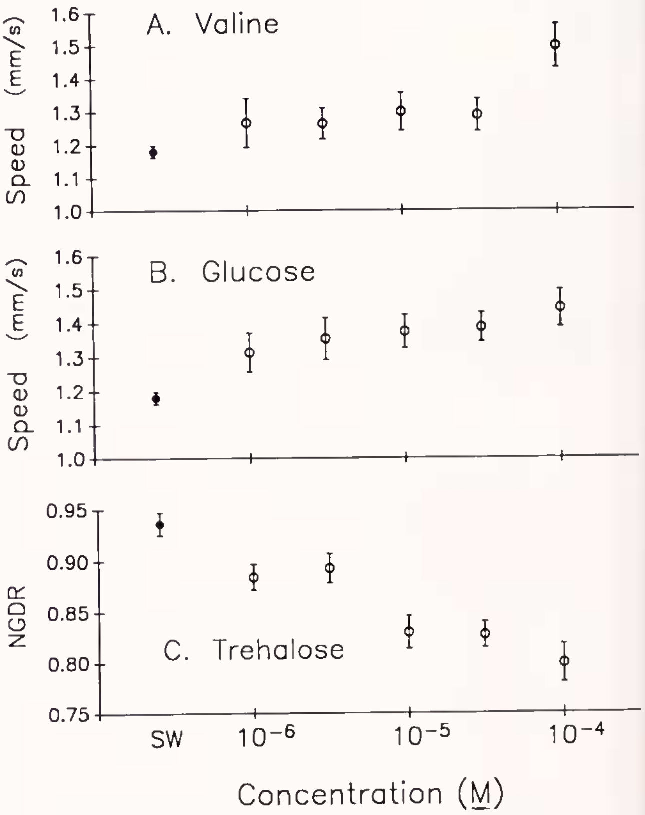

Figure 4. Dose-response curves for larvae exposed to test substances causing maximal responses. Each point gives the mean ± 1 SEM. Values for seawater controls (SW) at extreme left are shown for comparison, *n* for seawater = 79. A. Swimming speed in valine as a function of concentration, $F_{1,264} = 5.932$, $P < 0.025$; *t*-score testing for intercept different from seawater = 8.937, $n = 131$, $P < 0.001$. B. Speed in glucose as a function of concentration, $F_{1,295} = 3.65$, $P < 0.05$; *t*-score testing for intercept different than seawater = 45.881, $n = 155$, $P < 0.001$. C. NGDR in trehalose as a function of concentration, $F_{1,538} = 22.897$, $P < 0.001$; *t*-score testing for intercept different than seawater = 12.204, $n = 181$, $P < 0.001$.

Table II

Feeding responses by adult Uca longisignalis *to chemical test solutions and seawater controls*

	Proportion feeding		Feeding time	
Compound	Proportion	*G*-value	Time (s)	*t*-value
Amino acids				
β-alanine	0.17	7.33**	11 ± 2	4.59***
arginine	0.00	0.00		
asparagine	0.00	0.00		
cysteine	0.00	0.00		
glutamic acid	0.13	5.98*	28 ± 5	4.92***
glycine	0.00	0.00		
proline	0.00	0.00		
serine	0.17	7.33*	57 ± 23	2.51*
taurine	0.00	0.00		
valine	0.07	2.83		
Sugars				
galactose	0.00	0.00		
glucose	0.60	36.61***	841 ± 164	5.12***
maltose	0.80	43.71***	367 ± 63	6.91***
sorbose	0.20	8.22**	269 ± 108	2.48*
sucrose	0.60	36.61***	437 ± 86	5.91***
trehalose	0.33	10.97**	535 ± 81	6.63***
Seawater	0.00	—		

Thirty different crabs were tested with each compound and with seawater. Mean feeding time is given ±1 SEM. When there was no significant feeding as judged by a *G*-test, *t*-tests of feeding times were not performed. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

versus feeding time, yields a value of 0.58 ($0.03 < P < 0.06$). In spite of this association, the patterns of feeding induction (frequency or proportion) and feeding intensity (time) show qualitative differences. Trehalose, for instance, produced a feeding frequency little different from serine or glutamic acid, yet feeding times for animals exposed to trehalose rank second only to glucose (Table II). Maltose treatments exhibited the greatest ability to induce feeding, yet rank fourth in the intensity of the response (Table II).

Correlations among life-history stages, and among species

The correlations among adult and larval responses of *U. longisignalis* are based on the data given in Table III. Responses of the two life-history stages are similar at a very coarse scale, where a binomial test indicates that adults and larvae mutually respond either positively or negatively to the same substances more often than predicted by chance alone (binomial test: $n = 16$, $x = 12$, $P < 0.04$). However, the fine scale pattern of response exhibited by each life history stage differs substantially. A Kendall's Tau performed on ranks based on the magnitudes of larval and adult responses to the same compounds yields a value of −0.0227, indicating no significant correlation ($P > 0.50$). Behavior of larvae in trehalose is quite different than responses to the other compounds, making the functional significance of the response to trehalose unclear. Therefore, the correlation analysis was repeated with the larval response to trehalose ranked last, and also with this compound deleted from the analysis entirely. In both cases, the results are unchanged; Kendall's Tau,

Table III

Analysis of chemosensory responses between life-history stages in Uca longisignalis, *and between adults of* U. longisignalis *and* U. pugilator

	Larvae		Adults			
	U. longisignalis		*U. longisignalis*		*U. pugilator*	
Compound	Score	Rank	Score	Rank	No.	Rank
Valine	7.60	1	2.84	9[a]	—	—
Trehalose	4.31	2	10.97	4	20.3	5
Glucose	3.75	3	36.61	2.5	60.0	3
Sorbose	3.36	4	8.24	5[a]	—	—
Galactose	2.99	5	0.00	10[a]	—	—
β-alanine	2.86	6	7.33	6.5[b]	10.0	7
Sucrose	2.54	7	36.61	2.5	133.7	1
Maltose	2.14	8	43.71	1	85.5	2
Serine	0.79	9	7.33	6.5[b]	51.1	4
Glutamic acid	0.77	10	5.98	8[c]	10.0	7
Asparagine	—	—	0.00	8[d]	10.0	7

Score refers to the results of a *t*-test (larvae) or *G*-test (adults) for significant behavioral responses to the test compound. For *U. pugilator*, No. refers to the mean number of foodballs produced by individual crabs foraging on the test compound, with the data taken from Robertson *et al.* (1981). [a] Ranking for adult-larval comparison only, not used in adult-adult comparison. [b] Ranking for adult-larval comparison only, rank for adult-adult comparison is 5.5; [c] Ranking for adult-larval comparison only, ranking for adult-adult comparison is 7; [d] Ranking for adult-adult comparison only, compound not used in adult-larval comparison. See text for details.

computed with trehalose ranked last, is −0.3423, and is −0.2319 with trehalose removed ($P > 0.25$ in both cases).

The difference in fine *versus* coarse scale patterns reflects changes in the stimulatory capacity of substances excitatory to both life history stages. Only four compounds were significantly stimulatory exclusively to larvae or to adults (valine and galactose were highly excitatory solely to larvae, while glutamic acid and serine induced only adults to feed); the majority of the remaining compounds tended to be more highly excitatory to one stage or the other. Maltose and sucrose, for instance, were highly stimulatory to adults, but less stimulatory to larvae, while trehalose was more stimulatory to larvae than to adults.

In contrast to the within species comparison, the responses of adult *U. pugilator* and *U. longisignalis* are highly correlated at both coarse and fine scales. A binomial test indicates that adults from both species mutually respond either positively or negatively to the same compounds more frequently than expected by chance (binomial test: $n = 14, x = 13, P < 0.001$). Similarly, a Kendall's Tau, computed on the rankings in Table III, gives a value of 0.78 ($P < 0.03$), indicating a quantitatively similar response pattern to individual compounds among adults of each species. Although we present our tabulation using rankings of the proportion feeding for *Uca longisignalis*, the results using feeding time are not substantially different (Tau = 0.57, $0.03 < P < 0.06$). The monosaccharide glucose, its disaccharide isomers, and the amino acid serine were the most stimulatory, while the remaining amino acids were weakly stimulatory to both species.

Discussion

This is the first examination of patterns of chemoreception in zoea stage larvae. We have shown that brachyuran crab larvae possess chemosensory abilities, and that these abilities are present, although perhaps not fully developed, at a very early stage. Larvae respond to various amino acids and sugars with a change in swimming behavior. Such changes have previously been demonstrated as reliable indicators of chemosensitivity in a variety of zooplankters, such as copepods (Poulet and Ouellet, 1982; Buskey, 1984), tintinnids (Buskey and Stoecker, 1989), oyster larvae (Zimmer-Faust and Tamburri, in prep.), and ciliates (Levandowsky *et al.*, 1984).

Workers have interpreted the changes in swimming behavior as indicating that waterborne substances act to cue resource acquisition behavior, since changes often involve decreases in swimming speed and in NGDR, and an increased rate of turning (Buskey, 1984; Buskey and Stoecker, 1989; Zimmer-Faust and Tamburri, in prep.). Together, these changes have been viewed as 'adaptive' responses, allowing predators to remain in aggregations of potential prey, or enabling larval settlers to conduct site-restricted searches for microhabitats favorable to substrate colonization. Clearly, the increased turning behavior displayed by fiddler crab larvae in trehalose solutions might facilitate location of prey within patches, because the turning causes animals to loop back, focusing locomotory activity within a small area (*e.g.*, Koopman, 1980).

Paradoxically, increases in swimming speed and NGDR also seem to be associated with prey detection. In this study, *Uca* zoea responded to metabolites from *Artemia*, a known zooplankton prey species, with an increase in both speed and NGDR. Buskey (1984) determined that, when copepods are given diatom suspensions, swimming speed and NGDR decrease, whereas copepods exposed to diatom exudates alone, increase both speed and NGDR. In the absence of mechanical stimuli, these behaviors might allow animals to cover large areas in search of prey patches, whereupon encountering prey, paired mechanical and chemical stimuli would result in site-restricted behavior. Additional experiments are necessary to determine the functional roles of chemoreception and mechanoreception in mediating prey search by *Uca* zoea.

To some degree, substances stimulatory to larvae are those that signal the presence of potential food items. Zooplankton are the primary prey species of *Uca* larvae, which have been successfully reared only using various motile zooplankton (*e.g.*, Herrnkind, 1968; Christiansen and Yang, 1976; Rabalais and Cameron, 1983; Christy, 1989). The larvae lack setae necessary for capturing phytoplankton, instead pinning prey between the telson and anteroventral spine (Herrnkind, 1968). *Uca longisignalis* larvae respond to the amino acids valine and β-alanine. Although the response spectrum is narrower than that observed in other (adult) crustaceans (see reviews of Ache, 1982; Carr, 1988; Laverack, 1988), these two amino acids strongly signal the presence of fleshy prey. Beta-alanine constitutes as much as 12% of the free-amino acids released into the water by zooplankton, with both valine and β-alanine being consistently among the most abundant amino acids released into the surrounding environment by potential zooplankton prey (Webb and Johannes, 1967). Beta-alanine is generally one of the most abundant free amino acids present in flesh, and valine is quite often abundant, averaging over 5% of the free-amino acid pool (Cowey and Corner, 1964; Srinivasagam *et al.*, 1971). Zooplankton assemblages have 2–3 times the free amino acid concentration of valine and β-alanine as phytoplankton-dominated communities (Jeffries, 1969).

Some of the stimulatory sugars may also signal the presence of prey. Glucose is the major blood sugar present in animals, while trehalose is found in the hemolymph of some crustaceans (Telford, 1968). Both of these compounds evoke filter feeding responses in the planktivorous porcelain crab *Petrolisthes cinctipes* (Hartman and Hartman, 1977). There is little work done on the distributions of other sugars in seawater or in tissues of marine animals, although galactose and galactose derivatives found in seawater may originate from flagellates (Sakugawa *et al.*, 1985).

Adult *U. longisignalis* respond to a rather limited suite of the compounds tested, primarily carbohydrates, and stimuli that are more effective at inducing feeding also produce more intense feeding responses (Table II). Glucose and glucose dissacharide moieties are particularly potent stimuli. Contrary to what has been found in other studies, there is little evidence that disaccharides are more stimulatory than constituent monosaccharides (*e.g.*, Zimmer *et al.*, 1979; Robertson *et al.*, 1981; Trott and Robertson, 1984). Judging by both the induction as well as the intensity of the response, crabs respond more highly to glucose compounds than to other substances. Some additional sugars produce appreciable responses of one type or the other (*i.e.*, induction or intensity), but the joint function of the two components is not as great as for glucose and its dissacharides. Although a few amino acids, particularly serine, provoke responses, they have small effects both as inducers, and in terms of the response intensity.

The pattern of chemosensitivity suggests adult *U. longisignalis* are well constructed to allow efficient foraging in their natural habitat. Diatoms, bacteria, and, occasionally, blue-green algae are dominant flora of the mud-flats preferred by crabs. The primary storage product in diatoms is a glucan, and carbohydrates are primary constituents of the extracellular mucose sheaths of diatoms and blue-green algae (*e.g.*, Fogg, 1966). These sugars are often released into the environment by algal species (Fogg, 1966; Fogg *et al.*, 1973; Craigie, 1974; Darley, 1977; Paulsen *et al.*, 1978). Serine is the dominant amino acid of the free amino acid pool of diatoms (Parsons *et al.*, 1961), and it appears that adult fiddler crabs are unique in responding primarily to this amino acid (*i.e.*, Robertson *et al.*, 1981). Strong responses to carbohydrates and serine may be expected in light of their specificity as a cue to microalgal food resources.

The methodology of this study and that of Robertson *et al.* (1981) on the sand fiddler crab, *Uca pugilator*, are parallel enough to quantify the correlation among chemosensory abilities of the two species. The analysis indicates that, in at least this species pair, similar trophic ecologies have produced a broad concordance among patterns of adult chemosensitivity, even on a fine scale. The high correlation among the abilities of *Uca* species may be a function of the habitat occupied by adult fiddler crabs. For the semi-terrestrial *Uca*, molecules present in sediments where crabs forage are derived from a variety of sources, including microbial breakdown processes (Rittschof, 1980), and leakage from dead cells and decaying material (Kennish, 1986). High background levels of substances in sediments, and the large number of potential non-prey sources of stimulatory molecules, may make intertidal mudflats particularly "noisy" environments. Under these conditions, only a few substances most closely associated with the presence of food (*i.e.*, serine and carbohydrates) may be good signal carriers, forcing convergence among the properties of organisms occupying these habitats. Future studies on animals in subtidal, intertidal, and semi-terrestrial habitats are needed to fully discern the influence of habitat type on patterns of correlation among chemosensory abilities of related crustacean species.

In contrast to the between-species comparison, larval and adult *Uca longisignalis* express different patterns of chemosensory ability at the fine scale. It appears that differences in the chemical environment of the pelagic and benthic habitats, or differences in diet, may require that varying life-history stages respond disparately to similar suites of chemicals. A number of compounds stimulatory to one life-history stage of *U. longisignalis* are either nonstimulatory or only slightly stimulatory to the other stage. For instance, valine and galactose are excitatory only to larvae; serine and glutamic acid only to adults. Valine and serine are cues that are well-defined markers for prey types favored by a particular form, so it is perhaps not surprising that each substance is strongly stimulatory to only one life-history stage. The significance of glutamic acid and galactose to certain stages is less clear. Glutamic acid is not readily released into seawater by zooplankton (Webb and Johannes, 1967), but is an abundant constituent of the amino acids found in diatoms (Cheucas and Riley, 1969). Glucose appears to be unique in its ability to function as a cue for both larval and adult food sources, and is highly stimulatory to both life-history stages.

Patterns of larval and adult abilities must be cautiously interpreted, because it appears that some stimulants may produce responses that do not enhance the ability of the organism to detect prey. There is no evidence that the sugars sorbose, maltose, and sucrose are present in zooplankton, but they are intimately associated with adult food sources. It may be that detection of these substances by larvae has some function. However, in view of the close association of these substances with adult food, we tentatively hypothesize that the chemosensory responses of larvae to these sugars are incipient abilities. That is, sensitivity to these compounds by larvae may be the result of developmental processes required in providing sensitivity by adults.

While larval abilities appear partially a consequence of adult requirements, adult abilities do not seem to reflect larval needs. Substances which act as potential cues to larval, but not adult prey items (*e.g.*, valine), do not elicit adult feeding behaviors, while still evoking strong larval responses. Central neural processing is known to attenuate chemical signals, thereby reducing or eliminating behavioral responses, even though substances may evoke strong electrophysiological activity from receptor cells (Dethier, 1980; Derby *et al.*, 1985). It is sometimes observed that substances that produce action potentials from sensory cells do not always have detectable effects on behavior. Shepheard (1974) compared results of his electrophysiological study on responses by olfactory cells of *Homarus americanus*, to behavioral studies of McLeese (1970), and concluded that a variety of compounds with strong electrophysiological activity elicited little or no behavioral reactions. Working with *U. pugilator*, Vermeer (1981) has documented that valine and β-alanine are both extremely potent substances in electrophysiological assays, although valine is not an adult feeding stimulant, and β-alanine is only weakly so (Robertson *et al.*, 1981). We suggest this type of discordance between electrophysiological and behavioral responses in adults may be adaptive, serving to reduce or eliminate adult feeding reactions to substances that cue only larval food resources.

The conclusions reached as a result of our study must be regarded as working hypotheses, primarily because we did not assay the changes in chemosensory-mediated behavior continuously through ontogeny from zoea and megalopa stages, to the adult stage. Our aim is to call attention to the patterns we have observed, to help investigators interpret chemosensory abilities of other organisms. Additional studies on the development of chemoreceptive behavior in fiddler crabs and in other organisms will be needed to confirm the patterns we have detected. Further investigations also will be required to determine the conditions under which chemosensory abilities of early life-history stages are constrained by developmental processes and ecological requirements of later forms. While the ecological context of organisms may produce concordance among chemosensory abilities, it nonetheless appears that constraints arising from developmental processes may sometimes complement ecological pressures in determining the expression of chemosensory-mediated behavior.

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